REMARKS

Claims 1-3 and 5-13 are pending in this application. By this Amendment, claims 1 and 5-12 have been amended, claim 4 has been cancelled, and claim 13 has been newly added. A substitute specification has also been provided. Applicants submit that no new matter is presented by these amendments.

Objection to the Drawings

The drawings were objected to under 37 C.F.R. § 1.83(a) for failing to show any lines or graphs indicating actin polymerization as described in the specification. Applicants believe that the drawing sheets being referred to in the Office Action are the translations of the text found on Applicants' originally-filed drawings, which were in French, and showed lines and graphs indicating actin polymerization. Applicants are submitting herewith replacement drawing sheets that show the graphs provided in the French-language version of the application, using English-language translations of the legends on the graphs. Withdrawal of this objection is therefore respectfully requested.

Objections to the Specification

The listing of articles in the specification was objected to as not being a proper Information Disclosure Statement. Applicants have submitted herewith an Information Disclosure Statement listing the articles referenced in the "Bibliographic References" section of the specification. Applicants also note that the Examiner did not consider the published PCT applications that were cited in the Information Disclosure Statement that was filed on June 17, 2005, because no copies were provided. Applicants have therefore resubmitted these published PCT applications with the Information Disclosure

Statement. Applicants respectfully request that these articles and published PCT application be considered by the Examiner.

The specification was also objected to because the French word "tampon" is used repeatedly in place of the words "solution" or "buffer." In response to this objection, Applicants have submitted herewith a substitute specification, in which all instances of the word "tampon" are replaced with the word "solution."

Withdrawal of these objections is therefore respectfully requested.

Rejections under 35 U.S.C. § 112

Claims 6-12 were rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as their invention, for the reasons set forth on pages 4-6 of the Office Action. In order to overcome these rejections, Applicants have amended claims 6-10 and 12 as set forth above, and added new dependent claim 13. Withdrawal of these rejections is therefore respectfully requested.

Rejections under 35 U.S.C. §§ 102(b) and 103(a)

The outstanding Office Action rejected claims 1, 2, and 6 under 35 U.S.C. § 102(b) as allegedly being anticipated by Tellam et al. (1986). Claims 1-3 and 8-9 were rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Malicka-Blaszkiewicz et al. (1995). Claims 1-8 and 12 were rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Tellam et al. (1986) in view of Menu et al. (2002). Claims 1-3 and 8-11 were rejected under 35 U.S.C. § 103(a) as allegedly being

unpatentable over Malicka-Blaszkiewicz et al. (1995). Applicants respectfully traverse these rejections.

The Presently-Claimed Invention

The presently-claimed invention relates to methods of analyzing the tumor aggressivity of cancerous cells consisting of the measurement of the quantity of polymerized actin in the steady state in a lysate of the said cells, wherein the measurement of the quantity of actin in the steady state is carried out by static fluorescence polarization in the presence of actin monomers bound to a fluorochrome, the monomers being incorporated into the actin filaments (actin F) formed during the endogenous actin polymerization of the lysate. The presently-claimed invention also relates to methods of identifying molecules likely to have anti-cancer activity, methods of evaluating cancer cells to determine their invasiveness, oncogenicity, and sensitivity to cancer treatments, as well as kits for carrying out these methods.

Rejections Over Tellam et al. and Menu et al.

Claims 1, 2, and 6 were rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Tellam et al. (1986), and claims 1-8 and 12 were rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Tellam et al. (1986) in view of Menu et al. (2002).

The Office Action takes the position that Tellam et al. discloses a method for determining the nucleating activity in tumorigenic cells, wherein tumorigenic cells are lysed in non-denaturing conditions and centrifuged to remove cellular debris. Fluorochrome-labeled actin monomers are added to the lysate along with substances

necessary for the polymerization of endogenous actin and protection of the lysate proteins. The quantity of polymerized actin monomers incorporated into the actin filaments from the cell lysate of tumorigenic cells is measured, and is compared to the reference quantity of polymerized actin from non-tumorigenic cells. Tellam et al. is also cited for disclosing a cell re-suspension medium, substances necessary for the endogenous actin polymerization and protection of the lysate proteins, solutions of actin monomers labeled with a fluorochrome, and extracts of tumorigenic and non-tumorigenic cells and reaction buffers.

However, the Office Action admits that Tellam et al. fails to disclose or suggest a method wherein the quantity of polymerized actin corresponds to the sum of all of the F-form actin, wherein the measurement of the quantity of actin in the steady state is carried out by static fluorescence polarization in the presence of actin monomers bound to a fluorochrome which are added to the cellular lysate in a proportion ranging from 1/80th and 1/1600th in relation to the quantity of endogenous actin, the monomers being incorporated into actin filaments (F-actin) formed during endogenous actin polymerization of the lysate, wherein the method is used to evaluate the invasive character of the cells, or a kit including a cell re-suspension medium for the cell lyses, substances necessary for the endogenous actin polymerization and the protection of the lysate proteins, a solution of actin monomers bound to a fluorochrome, an actin polymerization solution, a general actin solution, and optionally the extracts of aggressive and nonaggressive reference cells.

Menu et al. is cited for allegedly disclosing a method of determining the tumor aggressivity of cancerous cells by measuring the quantity of polymerized F-actin in the steady state of the lysate of the cells, comparing the value of the quantity of

polymerized F-actin in the cancerous cells to a reference value of non-migrating cells, exposing the cells to the actin polymerization inhibitor latrunculin-A, and determining the capacity of the latrunculin-A to restore the quantity of polymerized actin in the steady state to that of the non-migrating cells. Menu et al. is also cited for allegedly disclosing that F-actin becomes polarized when the cells are migrating, in contrast to non-migrating cells, and that this polarization can be visualized by fluorescence and confocal laser scanning microscopy and quantified by fluorometry and flow cytometry.

Contrary to the disclosures of Tellam et al. and Menu et al., the presently-claimed methods relate to the measurement of the ***endogenous*** actin polymerization, not ***exogenous*** actin. Tellam et al. describes the measurement of the skeletal muscle actin, not endogenous actin. Thus, the presently-claimed methods allow actin polymerization to be measured under conditions like those found in the cell (i.e., *in vivo*).

Further, Applicants submit that neither of Tellam et al. and Menu et al. discloses the presently-claimed methods of measuring endogenous actin by static fluorescence polarization. The techniques used to measure actin polymerization in Tellam et al. and Menu et al. are limited to fluorescence microscopy, confocal laser scanning microscopy, fluorometry, and flow cytometric analysis. Use of static fluorescence polarization is not disclosed or suggested by either of Tellam et al. or Menu et al. Moreover, as is discussed in the "Comments Regarding Fluorescence Polarization" section below, it would not have been obvious to one skilled in the art at the time the present invention was made to measure actin polymerization using static fluorescence polarization.

In view of the amendments and remarks above, Applicants respectfully submit that Tellam et al. and Menu et al., alone or in combination, fail to disclose or suggest the features of the pending claims. As such, Applicants respectfully request that the rejections of claims 1-3 and 5-13 as allegedly being anticipated by and/or unpatentable over any of Tellam et al. and Menu et al. be withdrawn.

Rejections over Malicka-Blaszkiewicz

Claims 1-3 and 8-9 were rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Malicka-Blaszkiewicz et al. (1995), and claims 1-3 and 8-11 were rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Malicka-Blaszkiewicz et al. (1995).

The Office Action takes the position that Malicka-Blaszkiewicz et al. discloses a method wherein the steady-state quantity of polymerized F-actin in cancerous cells is compared with the steady-state quantity in a control cell lysate. The cells are lysed in non-denaturing conditions, centrifuged to remove cellular debris, suspended in a buffer necessary for endogenous actin polymerization and the protection of lysate proteins, and the quantity of polymerized actin in the steady state of the lysate between the cancerous and control cells is compared.

Malicka-Blaszkiewicz et al. is also cited for disclosing that a high level of actin polymerization is a prerequisite for the formation of pseudopodia, which in turn are necessary for the infiltration of cells into tissues (invasion) and eventually for efficient metastasis formation (oncogenicity). The Office Action further alleges that Malicka-Blaszkiewicz et al. discloses that actin is a possible marker in the evaluation of the stage of tumor growth and its metastatic potential, and could be expected to pave the

way for therapeutic intervention, aimed at stopping and possibly reversing the process of metastatic growth with the use of drugs affecting actin polymerization.

Applicants submit that Malicka-Blaszkiewicz et al. does not disclose a method of measuring endogenous actin by static fluorescence polarization. The technique used to measure actin polymerization in Malicka-Blaszkiewicz et al. is limited to the inhibition of DNase 1 from bovine pancreas. It appears that the Office Action recognizes this distinction, as previously-submitted claim 4 (the subject matter of which has now been incorporated into independent claim 1) was not rejected over Malicka-Blaszkiewicz et al.

In view of the amendments and remarks above, Applicants respectfully submit that Malicka-Blaszkiewicz et al. fails to disclose or suggest the features of the pending claims. As such, Applicants respectfully request that the rejections of claims 1-3 and 5-13 as allegedly being anticipated by and/or unpatentable over Malicka-Blaszkiewicz et al. be withdrawn.

Comments Regarding Fluorescence Polarization

Fluorescence anisotropy (polarization) is a technique specially applied to study molecular interactions. It gives a direct, nearly instantaneous measure of a tracer's bound/free ratio. Time-resolved fluorescence anisotropy allows detection of low protein concentrations (i.e., equilibrium analysis into the low picomolar range) and is a rapid and reliable way to measure equilibrium binding for biological molecules including proteins and drugs.

Anisotropy measurements are based on the principle of photoselective excitation of fluorophores by a polarized light leading to a subsequent polarized emission (high anisotropy). Fluorescence polarization (anisotropy) has been used to study molecular

interactions by monitoring changes in the apparent size of fluorescent-labelled or inherently-fluorescent molecules. When a small molecule (tracer) is excited with plane polarized light, the emitted light is largely depolarized because the molecule rotates rapidly in solution during the fluorescence life time (time between excitation and emission). However, if the tracer is bound to a larger receptor molecule, thereby increasing its effective molecular volume, its rotation is slowed sufficiently to emit light in the same plane in which it was excited. The bound and free states of the tracer each have a fluorescence polarization value, a high value for the bound state and a low value for the free state. The measured polarization is an average of these two values, thus providing a direct measure of the fraction of tracer bound to receptor.

Fluorescent-labelled cellular lysate actin filaments may be acted upon by a drug or a protein that cleaves them into smaller fragments. The lysate actin filaments have a high anisotropy value (Δ mA max), while the smaller fragments, which rotate faster than the filaments, necessarily have lower anisotropy values. In this way, it is possible to study the effect of drugs that protect actin filaments from cleaving by cofilin, an actin associated protein whose expression is increased in some tumoral cells, which disrupts actin organization.

When measuring the anisotropy of fluorochrome-bound actin in the absence of cellular extract, the labelled actin is free to rotate in solution (low anisotropy value). When the fluorochrome-bound actin is incorporated into actin filaments from cellular extract, the large complex is slow to rotate in solution (high anisotropy value). The addition of drugs that interact with cellular lysate actin filaments and modulate actin polymerization (K_{obs}) increase anisotropy values at equilibrium (Δ mA max). It is therefore possible to directly observe that a drug interacts with actin by measuring the

increase in anisotropy values in solution. However, rotational diffusion or flexibility of the fluorophore also represents a major cause of depolarization of light (low anisotropy value). Accordingly, any increase in polarization that may be observed in solution cannot be directly linked to the rotation of the actin polymer, because changes in the flexibility of the tracer incorporated into the actin filaments may also be responsible for the increase.

In the methods of the presently-claimed invention, the time course of actin polymerization is observed in cell extracts, not purified protein solutions. In accordance with the development of the methods of the presently-claimed invention, the inventors discovered that the flexibility of fluorochrome-bound actin monomers decreases when they are incorporated in actin filaments during the endogenous actin polymerization of the lysate. Accordingly, the polarization increase of the fluorescent tracer is measured when it is incorporated in pre-formed actin filaments from cell extracts.

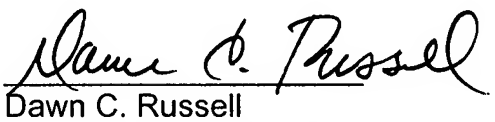
CONCLUSION

In view of the foregoing, reconsideration of the application, withdrawal of the outstanding rejections, allowance of Claims 1-3 and 5-13, and the prompt issuance of a Notice of Allowability are respectfully solicited.

Should the Examiner believe anything further is desirable in order to place this application in better condition for allowance, the Examiner is requested to contact the undersigned at the telephone number listed below.

In the event this paper is not considered to be timely filed, Applicants respectfully petition for an appropriate extension of time. Any fees for such an extension, together with any additional fees that may be due with respect to this paper, may be charged to counsel's Deposit Account No. 01-2300, **referencing Attorney Dkt. No. 021305-00214.**

Respectfully submitted,



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